THE SODIUM-POTASSIUM EXCHANGE PUMP

II. Analysis of Na^+-Loaded Frog Sartorius Muscle

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ABSTRACT A model for the Na-K exchange pump was applied to data on Na^+-loaded frog sartorius muscle, and was used to relate the rate of adenosine triphosphate (ATP) hydrolysis to the electrical properties of the cell membrane. Membrane hyperpolarization was considered to arise from an electrical current which was produced by the hydrolysis reaction coupled to ion movements, and which flowed across the membrane. The reaction rate, as calculated from hyperpolarization, agreed with direct measurements of ATP hydrolysis and with the rate estimated from Na^+ tracer efflux studies. Although Na^+ is actively extruded, the model showed that K^+ is inwardly transported if the potassium permeability of the membrane is less than about 6.6×10^{-6} cm/sec, as is suggested by resistance data. Calculations indicated that the reaction conductance L_{rr} was relatively constant when compared with the reaction rate and reaction free energy for large changes in internal and external ionic concentrations. Its value agreed with the value obtained from the dependence of Na^+ tracer efflux on external K^+. A set of experiments was suggested which would provide a more complete interpretation of the data.

INTRODUCTION

A model of the Na-K exchange pump in which membrane potential is regulated by the reaction rate of the pump, and the rate in turn depends on membrane potential, was developed in part I of this study (Rapoport, 1970) and is summarized in the Appendix. The model will be employed to analyze data on frog sartorius muscle, and the extent of its applicability and limitations will be discussed.

Keynes (1954) proposed that the increase of Na^+ tracer efflux in frog sartorius, when the external K^+ was increased, was due to a coupled Na-K exchange pump. He defined the pump as "electrogenic" when membrane potential (inside-outside) was more negative (hyperpolarized) than the K^+ equilibrium potential. This occurs in Na^+-loaded frog sartorius muscle in 10 mM K^+ Ringer (Stephenson, 1953; Kernan, 1962; Mullins and Awad, 1965; Adrian and Slayman, 1966; Cross et al., 1965). The latter authors, who measured ionic concentrations and membrane po-

tentials with time in the Na^+-loaded muscles, did not propose a general formulation to interpret their results. Alternatively, rather than being due directly to the electrogenic pump, the hyperpolarization in Na^+-loaded muscles could be caused by K^+ being pumped into the muscle faster than it was replenished in the extracellular space by diffusion from the bathing solution (cf. Page and Storm, 1965; Adrian and Slayman, 1966).

The model will be used to show that in Na^+-loaded muscle, membrane hyperpolarization, which we define as the difference between the membrane potential and the "diffusion" potential, can be ascribed to the electrogenic pump. Reaction rate J_r, reaction conductance L_{rr}, the driving force on the reaction ΔF_r, membrane resistance, and other parameters will be calculated. J_r and L_{rr} will be shown to agree with values found by other means, and their relation to changes in internal and external concentrations will be considered.

A similar theoretical approach to Na^+-loaded frog sartorius has been made by Frumento (1965), but he did not account for the dependence of the pump on membrane potential and did not propose a feedback relation between pump rate and membrane potential.

METHODS

Assumed Parameters and Given Conditions

Cross et al. (1965) exposed pairs of frog sartorius muscles at 2°C to a K^+-free soaking-in solution which consisted of 89 mM NaCl, 25 mM $NaHCO_3$, 3 mM NaH_2PO_4, 0.9 mM $CaCl_2$, 1.5 mM $MgSO_4$, equilibrated with 5% CO_2–95% O_2. In this solution, the Na^+ content of the muscles increased and the K^+ content decreased; the muscles became "Na^+-loaded". After soaking, one muscle was transferred to a recovery solution containing 10 mM K^+ (Table I) and the K^+ and Na^+ contents of the other determined by flame photometry. In the recovery solution, the membrane potential of the one muscle was measured over a 1 hr period, after which its K^+ and Na^+ contents were measured. The combined values of ϕ_s and $\phi_K = RT/F \ln C^1_K/C^2_K$ (K^+ equilibrium potential, where superscripts *1* and *2* denote bathing solution and inside, respectively) initially and after 1 hr in recovery solution are listed in Table I for the muscle pairs, which were rank-ordered from 1 to 36 as a function of the initial potential differences $\phi_s - \phi_K$.

We assume that inside concentrations C^2_i change exponentially with a time constant τ of 30 min (Desmedt, 1953; Cross et al., 1965). If the initial concentration is $(C^2_i)_{t=0}$ and the final concentration is $(C^2_i)_{t=\infty}$, then

$$(C^2_i)_t = (C^2_i)_{t=\infty} + ([C^2_i]_{t=0} - [C^2_i]_{t=\infty}) \exp(-t/\tau). \qquad (1\,a)$$

Differentiating to obtain dC^2_i/dt and noting that the volume to surface ratio of the muscle cylinder is $r/2$ (where r is the fiber radius), the net flux of substance i per cm^2 surface is given by

$$J_{net,i} = (r/2\tau)([C^2_i]_{t=\infty} - [C^2_i]_{t=0}) \exp(-t/\tau). \qquad (1\,b)$$

TABLE I

A COMPARISON OF ϕ_s AND ϕ_K AT THE BEGINNING AND AFTER 1 HR OF RECOVERY*

Muscle No.	Initial			1 hr		
	ϕ_s	ϕ_K	$\phi_s - \phi_K$	ϕ_s	ϕ_K	$\phi_s - \phi_K$
	mv	*mv*	*mv*	*mv*	*mv*	*mv*
1	−67	−49	−18	−67	−65	−2
2	−76	−60	−16	−62	−65	3
3	−43	−27	−16	−70	−60	−10
4	−55	−40	−15	−60	−59	−1
5	−72	−58	−14	−65	−68	3
6	−70	−59	−11	−65	−67	2
7	−69	−60	−9	−54	−68	14
8	−68	−60	−8	−67	−68	1
9	−68	−61	−7	−66	−66	0
10	−62	−56	−6	−61	−63	2
11	−64	−59	−5	−66	−64	−2
12	−58	−53	−5	−65	−67	2
13	−65	−61	−4	−66	−66	0
14	−52	−48	−4	−57	−63	6
15	−63	−60	−3	−63	−67	4
16	−51	−48	−3	−70	−62	−8
17	−60	−59	−1	−53	−66	13
18	−24	−27	3	−44	−55	11
19	−44	−48	4	−53	−64	11
20	−28	−34	6	−60	−61	1
21	−43	−50	7	−64	−65	1
22	−40	−47	7	−60	−68	8
23	−39	−47	8	−50	−60	10
24	−39	−48	9	−57	−59	2
25	2	−8	10	−49	−49	0
26	−32	−43	11	−49	−58	9
27	−28	−43	15	−48	−59	11
28	−28	−43	15	−50	−61	11
29	−13	−28	15	−19	−57	38
30	−19	−37	18	−40	−51	11
31	−29	−50	21	−44	−61	17
32	−26	−49	23	−50	−68	18
33	−22	−47	25	−39	−64	25
34	−22	−49	27	−33	−60	27
35	−22	−50	28	−34	−62	28
36	−17	−54	37	−42	−65	23

C^i_K = 10 mM; C^i_{Na} = 104 mM; C^i_{Cl} = 85 mM.

$C^{\bar{s}}_{Na}$ (initial) = 97 mM; $C^{\bar{s}}_{Na}$ (1 hr) = 43.3 mM.

* From Cross et al., 1965.

The Na^+ content of the individual muscles was not reported. The reported mean initial Na^+ content was 80.5 mM Na^+/kg wet weight (measured on muscle in K^+-free soaking-in solution) and 38.8 mM/kg wet weight after 90 min in recovery solution. Respective mean water contents were 799 and 784 ml/kg wet weight. Fiber water and Na^+ concentration/liter

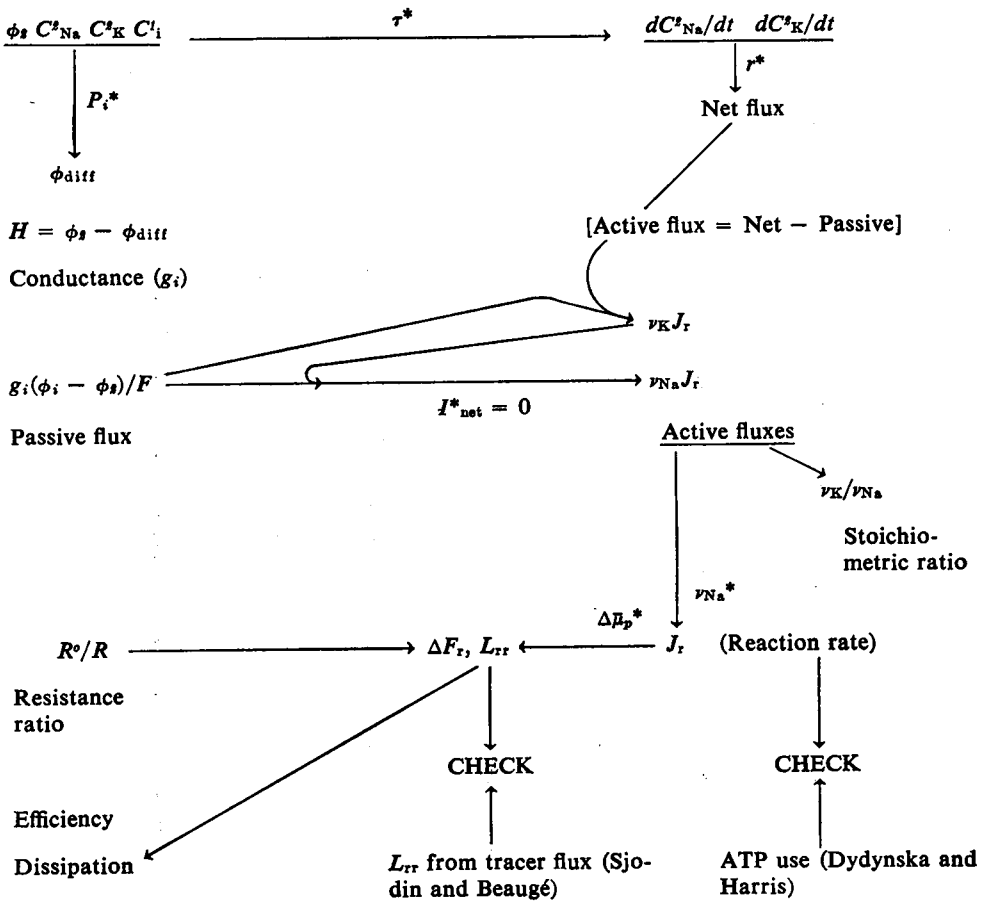

FIGURE 1 Flow chart for analysis of data on Na^+-loaded frog sartorius in 10 mM K^+ Ringer (Cross et al., 1965). Given quantities are on first line at left. Arrows point to calculated quantities. (*) indicates additional parameters required for calculation. CHECK shows quantities that can be compared with other data. For example, the reaction rate J_r calculated by the model can be compared with the rate of ATP hydrolysis as found by Dydynska and Harris (1966).

fiber water were calculated as shown by Cross et al. (1965), using an extracellular space of 130 ml/kg wet weight. With use of equation 1 *a* and the values above, average internal sodium concentration was C^s_{Na} = 97 mM initially and 43.3 mM at 1 hr.

Table I lists the concentrations and potentials for the initial and 1 hr conditions of Cross et al. (1965). Fiber radius r was taken as 40 μ. Although calculations require a choice of P_K, its exact value is not known. It may be constant, a function of only membrane potential, or of the difference $\phi_s - \phi_K$ (Hodgkin and Horowicz, 1959; Freygang et al., 1964); there is no indication that in muscle it depends on reaction rate. Complete analyses by the model were made with two assumptions about P_K : (*a*) it is constant and equals 1.5×10^{-6} cm/sec, a value assumed by Freygang et al. (1964) at ϕ_s = −84 mv, and (*b*) its value is such that all K^+ movement can be ascribed to passive diffusion (ν_K = 0). The question of active K^+ transport was considered in the light of these analyses. In the calculations $P_{Na} = 0.01\ P_K$

and $P_{Cl} = 3 \times 10^{-6}$ cm/sec (Hodgkin and Horowicz, 1959; Freygang et al., 1964). To obtain C^2_{Cl}, it first was estimated for the initial and 1 hr conditions by letting $\phi_{Cl} = \phi_s$ (Hodgkin and Horowicz, 1959). $(C^2_{Cl})_{t=\infty}$ then was calculated by equation 1 *a* when $\tau = 30$ min, and $J_{net,Cl}$ was found by equation 1 *b*. Using these values, an improved estimate of C^2_{Cl} was calculated by equation A 5. Since the new estimated value differed only slightly from the original one, it and $J_{net,Cl}$ were not recalculated.

The free energy of hydrolysis of ATP, $\Delta\bar{\mu}_p$, was taken as −48,000 joules, using estimated internal concentrations of ATP, adenosine diphosphate (ADP), and P_i (Kushmerick, 1969). Calculations were done with the use of the GE Mark I time-sharing computer (General Electric Co., Information Devices Dept., Oklahoma City, Okla.).

Fig. 1 represents the flow chart for calculations. Membrane potentials and concentrations were provided in Table I. Changes in concentrations with time were calculated by equation 1 *a* using the time constant τ and radius r, and net ionic fluxes were calculated by equation 1 *b*. With the assumed permeabilities P^*_i and concentrations C_i (where * represents an assumed parameter), the diffusion potential ϕ_{diff}, and H, the difference between the diffusion potential and ϕ_s, were calculated by equations A 6 and A 7. In addition, ionic passive fluxes due to diffusion and ionic chord conductances g_i were calculated by equation A 5. Since net ionic flux is the sum of passive and active terms, subtraction of the passive from the net term should give the active term, which is how $J_{active,K}$ was obtained. $J_{active,Na}$ was calculated instead by equation 2 for the constraint $I_{net} = 0$ (see Results). Flux and current are defined as positive in the direction from *1* to *2*, i.e., from outside to inside of the cell. Thus, $J_{active,Na} = -\nu_{Na}J_r$ and $J_{active,K} = \nu_K J_r$, since Na^+ is pumped outward. The ratio

TABLE II

MEMBRANE POTENTIALS AND IONIC CONCENTRATIONS IN Na^+-LOADED MUSCLES UNDER DIFFERENT CONDITIONS

Condition	ϕ_s	C^1_K	C^2_K	C^1_{Na}	C^2_{Na}	C^1_{Cl}	ϕ_K	$\phi_s - \phi_K$
	mv	*moles/liter*	*moles/liter*	*moles/liter*	*moles/liter*	*moles/liter*	*mv*	*mv*
A	−76.8	5	88.1	120	31.9	105	−72.3	−4.5
B	−69.9	10	82.7	120	37.2	110	−53.3	−16.6
C	−77	5	65.3	120	54.7	0*	−64.8	−12.2
D	−72.5	10	103.1	120	25	0*	−58.8	−13.7
E	−80	10	95	109.4	52	120.6	−56.7	−23.3
F	−80	10	80	109.4	70	120.6	−52.4	−27.6
G	−80	10	18	109.4	125	120.6	−14.8	−65.2
H	−118	1	92	118.4	45	120.6	−113.9	−4.1
I	−108	2.5	92	116.9	45	120.6	−90.9	−17.1
J	−80	10	92	109.4	45	120.6	−55.9	−24.1
K	−60	25	92	94.4	45	120.6	−32.8	−27.2
L	−25	100	92	19.4	45	120.6	2.1	−27.1

Values in conditions A–D are taken from Harris and Ochs (1966) and are means of more than one muscle pair. The solutions of A and D contained 20 mM HCO_3^-. The C^2_{Na} were estimated by assuming $C^2_K + C^2_{Na} \simeq$ 120 mM (cf. Adrian and Slayman, 1966). E–L represent means from Martirosov and Mykaelian (1970). The muscles in E–G had soaked for 24, 48, and 74 hr respectively in K^+-free solution, which changed the internal ionic concentrations. H–L have different external K^+ (and Na^+) concentrations, but internal concentrations are constant.

* Chloride replaced by methane sulfonate anion.

of active fluxes is the stoichiometric ratio ν_K/ν_{Na}. The constraint $\nu_K = 0$ means that $J_{net,K} = J_{passive,K}$, so that P_K for this condition was calculated from $J_{net,K}$, ϕ_s, and ionic concentrations by equation A 5.

Na^+ efflux kinetics suggest that $\nu_{Na} = 3$ (Mullins and Frumento, 1963), and a value between 2 and 3 is found in many tissues (Caldwell, 1968), which led us to choose ν_{Na} equal to 2 or 3 in the calculations. The model does not require that the ν_i be the same for different experimental conditions, and changes in ν_i could account for nonlinearities and changes in calculated pump parameters when the experimental conditions changed (see Discussion, equation 3). Reaction rate was calculated from $-\nu_{Na}J_r$ and compared to the rate of ATP hydrolysis (Dydynska and Harris, 1966). Using the assumed value of $\Delta\bar{\mu}_p$, ΔF_r was calculated by equation A 3 and then L_{rr} by equation A 2 as the ratio $J_r/(-\Delta F_r)$. L_{rr} was also obtained, as will be shown, from tracer flux measurements and compared with the calculated value. Membrane resistance R, resistance ratio R^o/R, and efficiency were calculated by equations A 9–A 11.

In addition to analyzing data of Cross et al. (1965), the relations of J_r, L_{rr}, and R to ionic concentrations were estimated by the model from data of Martirosov and Mykaelian (1970) (Table II). These authors did two sets of experiments. In conditions E–G of Table II, muscles were left in K^+-free soaking-in solution for 24, 48, and 72 hr respectively, so as to study membrane potential as a function of internal concentrations. In conditions H–L, outside K^+ was changed with constant internal concentrations. Since conditions E–L are "initial" condition experiments, net ionic fluxes could not be calculated from them. The data of Harris and Ochs (1966) (A–D of Table II) were used to analyze membrane resistance in Cl^--containing and Cl^--free media, so as to estimate P_K by equations A 9 and A 10.

RESULTS

Data of Cross et al. (1965) were analyzed following the flow sheet of Fig. 1, and some calculated means are listed in Table III. Fig. 2 *a* relates $H = \phi_i - \phi_{diff}$

TABLE III

CALCULATED MEAN PARAMETERS FOR MUSCLES 1–17 OF TABLE I

Parameters	Units	$P_K = 1.5 \times 10^{-6}$ cm/sec		P_K at $\nu_K = 0$*
		Initial	1 hr	Initial
H	mv	−4.2	−0.6‡	−7.6
ΔF_r	joules × 10^3	−31 (36)§	−20 (29)	−29
L_{rr}	moles/sec per cm² per joule × 10^{-16}	6.0 (7.6)	2.4‡ (0.9)	8.7
J_r	pmoles/cm² per sec	18.9 (28.4)	1.7 (2.5)	26.2
$FJ_r(\nu_K - \nu_{Na})$	μamp/cm²	−1.51	−0.14‡	−7.57
ν_K/ν_{Na}		0.70	0.83‡	0
R	kohm cm²	2.8	2.6	1.2
R^o/R		0.99	0.89 (0.96)	0.81
Efficiency		0.4 (0.3)	0.6 (0.4)	0.4

* Median value of P_K at $\nu_K = 0$ is 6.6×10^{-6} cm/sec.

‡ Not significantly different from 0 ($P > 0.05$).

§ Mean within parentheses is for $\nu_{Na} = 2$ if it differs from mean for $\nu_{Na} = 3$, which is outside of parentheses.

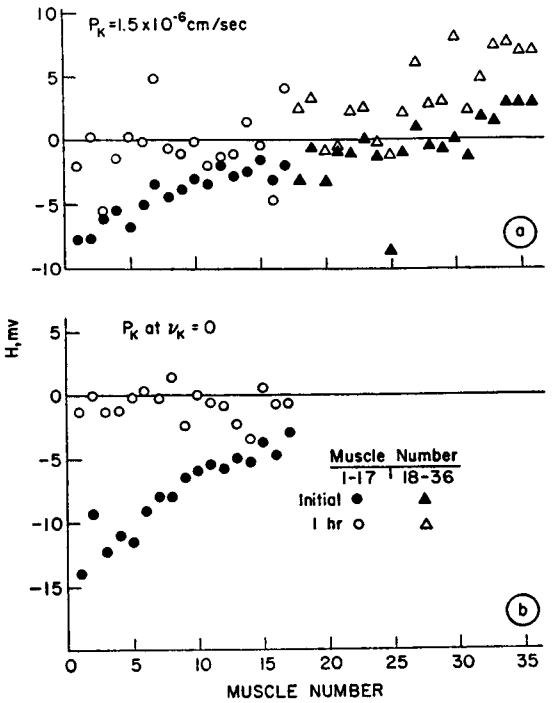

FIGURE 2 Relation of H to muscle number for $P_K = 1.5 \times 10^{-6}$ cm/sec and for P_K at $\nu_K = 0$. Results for the latter permeability are shown only for muscles 1–17, when $\phi_g < \phi_K$ initially. Means of H are in Table III.

(equation A 7) to muscle number when $P_K = 1.5 \times 10^{-6}$ cm/sec, where ϕ_g is from Table I and ϕ_{diff} calculated by equation A 6. Most of the muscles appear hyperpolarized initially, although in Table I, $\phi_g - \phi_K$ may be as positive as +34 mv. $\phi_g - \phi_K$ as a definition for H (cf. Kernan, 1962; Cross et al., 1965) was not used because if K^+ is transported actively ($\nu_K \neq 0$) or if the muscle is not at a stationary state, K^+ would not be expected to be in equilibrium with the diffusion potential i.e., $\phi_{diff} \neq \phi_K$ (Rapoport, 1970). Fig. 2 *b* shows H for muscles 1–17 for P_K at $\nu_K = 0$.

The 36 muscles in Table I were rank-ordered and divided into two groups, 1–17 and 18–36. Cross et al. (1965) stated that muscles 3 and 18–36 had been treated with soaking-in solutions containing little or no calcium or had been examined during a cold period which might have damaged the frogs, and usually had membrane potentials more positive than −50 mv in the soaking-in solution. These muscles, whose membrane potentials also were lower than found by Adrian and Slayman (1966), probably were abnormal. For this reason, and because calculated $J_{passive, Na}$ may be incorrect when ϕ_g is much more positive than −50 mv (see below), results on muscles 18–36 were not tabulated. Inclusion of muscle 3 in the calculations, because of its value of $\phi_g - \phi_K$, did not change the results.

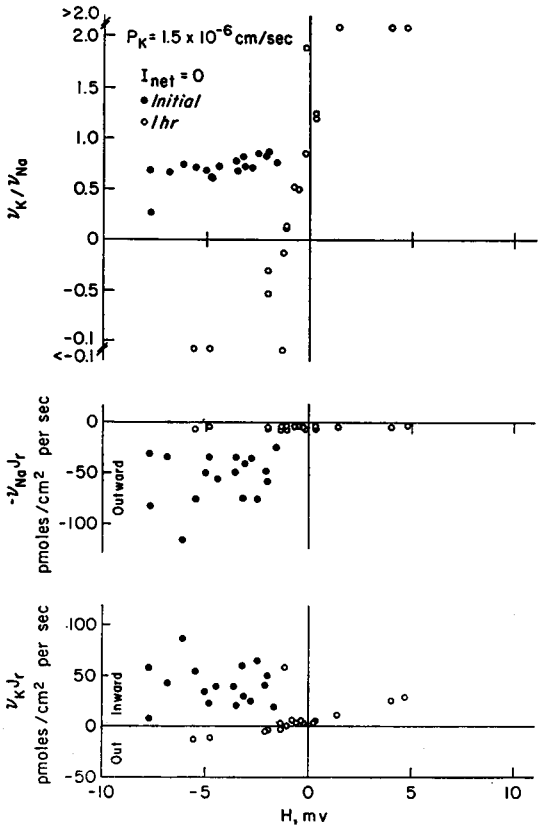

FIGURE 3 Relation for muscles 1–17 of active fluxes and ν_K/ν_{Na} to H. $P_K = 1.5 \times 10^{-6}$ cm/sec. Active K^+ flux was calculated as the difference between net K^+ flux (equation 1 *b*) and passive K^+ flux (equations A 4, A 5). Active Na^+ flux was calculated by equation 2 for the constraint $I_{net} = 0$.

Since current is not passed across the membrane, the condition $I_{net} = 0$ always should apply. Calculations did not yield this result when $J_{net,\,Na}$ and therefore $J_{active,\,Na}$ were found by equation 1 *b*, probably because the mean $\bar{C}^{\phi}_{Na}$ rather than the individual value for each muscle pair was used in that equation. In the analysis, $J_{active,\,Na}$ was calculated instead by the following equation, derived from equation A 4, in which it is first assumed that $I_{net} = 0$,

$$J_{active,\,Na} = -\nu_{Na} J_r = -\sum_i I_{passive,\,i}/F - \nu_K J_r \,. \tag{2}$$

In equation 2, the $I_{passive,\,i}$ (i = Na^+, K^+, and Cl^-) were calculated by equation A 5 and $J_{net,\,K}$ by equation 1 *b* in order to find $\nu_K J_r$. The results of the calculation of $J_{passive,\,Na}$ are relatively independent of inaccuracies in $\bar{C}^{\phi}_{Na}$ when $\phi_g < -50$ mv, because then $\bar{C}^{\phi}_{Na}$ in equation A 5 is multiplied by a factor < 0.14.

For $P_K = 1.5 \times 10^{-6}$ cm/sec, the calculated ionic active fluxes and the stoichi-

ometric ratios of muscles 1–17 are plotted in Fig. 3 for the condition $I_{net} = 0$. Active Na^+ flux $-\nu_{Na}J_r$ is outward and active K^+ flux $\nu_K J_r$ is inward initially. At 1 hr, these active fluxes are close to zero.

Table III gives the means of the calculated parameters for muscles 1–17 for the two P_K's and $\nu_{Na} = 3$ and 2. Many of the 1 hr means for $P_K = 1.5 \times 10^{-6}$ cm/sec and P_K at $\nu_K = 0$ did not differ significantly from zero. Since H also did not differ from zero, the pump would not contribute to membrane potential according to the model, and the pump parameters cannot be derived from it. A set of 1 hr values is included in Table III for illustrative purposes.

Dependence of Calculated $J_{active,\,K}$ on Estimate of P_K

Fig. 4 shows the calculated P_K's for muscles 1–17 when K^+ fluxes were assumed to be due only to passive diffusion ($\nu_K = 0$). The median value of these points is

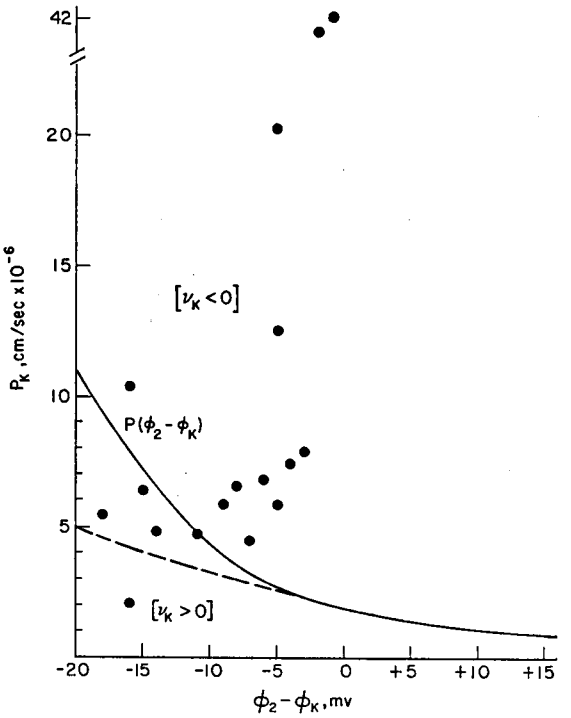

FIGURE 4 Relation of P_K to $\phi_s - \phi_K$. The solid curve represents our fit to the data of Hodgkin and Horowicz (1959) and is defined as the function $P(\phi_s - \phi_K)$. It is extrapolated from their data for $\phi_s - \phi_K < -16$ mv, or $P(\phi_s - \phi_K) > 8 \times 10^{-6}$ cm/sec to a maximum value of 15×10^{-6} cm/sec. The dashed line represents an alternative fit to the same data (Freygang et al., 1964). The points represent P_K for muscles 1–17 (Table I) at $\nu_K = 0$ (no active K^+ transport). Lower values would fall in the region for which $\nu_K > 0$ (K^+ is pumped inward). Higher P_K's predict that $\nu_K < 0$, which is unlikely (see text). The median value of the points is 6.6×10^{-6} cm/sec. The points overlap the function defined as $P(\phi_s - \phi_K)$ (continuous line).

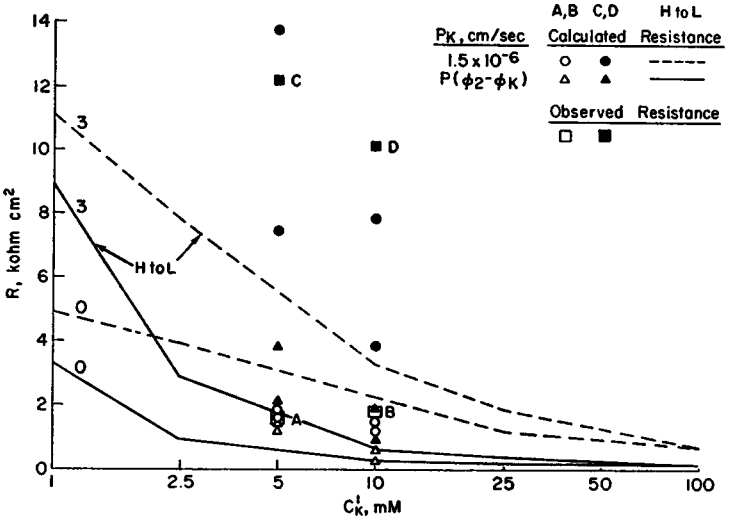

FIGURE 5 Relation of calculated and observed resistances to external K^+. The lines connect calculated R^o for H–L, when $P_K = 1.5 \times 10^{-6}$ cm/sec (dashed lines) and $P(\phi_2 - \phi_K)$ (continuous lines). For either permeability, the upper line shows $R^o = R$ (equation A 9) of an electroneutral ($\nu_{Na} = \nu_K$) or absent ($\nu_{Na} = \nu_K = 0$) pump. The lower line shows R^o when $\nu_{Na} = 3$ and $\nu_K = 0$, as calculated by equations A 9 and A 10. The ν_K are the numbers on the left-hand side of the figure. The square symbols are the observed resistances R^o in conditions A–D. P_{Cl} was taken as 7×10^{-6} cm/sec in A–D (Harris and Ochs, 1966). Pairs of open circles and triangles show calculated R^o for A and B, pairs of filled circles and triangles for C and D. The upper symbol of each pair gives R^o for the electroneutral pump, the lower symbol when $\nu_{Na} = 3$ and $\nu_K = 0$. P_K for A–D appears closer to 1.5×10^{-6} cm/sec than to $P(\phi_2 - \phi_K)$.

6.6×10^{-6} cm/sec. If the actual P_K's fall below the points in the figure, then the model indicates that K^+ is pumped inward to some degree and that $\nu_K > 0$. The region above the points would represent outward K^+ pumping, which is improbable (see Discussion).

Since an accurate estimate of P_K is unavailable, we will consider several possibilities. The solid line in Fig. 4 represents the function of $\phi_2 - \phi_K$, defined as $P(\phi_2 - \phi_K)$, which we estimated from data of Hodgkin and Horowicz (1959). It is extrapolated when $\phi_2 - \phi_K < -16$ mv, and agrees with the curve given by Frumento (1965). The dashed line represents an alternative dependence estimated by Freygang et al. (1964). It is possible that P_K depends only on membrane potential (Hodgkin and Horowicz, 1959; Freygang et al., 1964), in which case it would have a value less than 1.5×10^{-6} cm/sec for the initial potentials of muscles 1–17.

Fig. 4 shows that K^+ would be expected to be pumped inward initially ($\nu_K > 0$) if $P_K = 1.5 \times 10^{-6}$ cm/sec, if P_K is a function of potential alone, or if P_K is given by the dashed line in the figure. The function $P(\phi_2 - \phi_K)$ overlaps the points of P_K for $\nu_K = 0$, and represents permeabilities for which K^+ would only move pas-

sively. As expected, the mean ν_K calculated with $P(\phi_g - \phi_K)$ was not significantly different from zero initially for fibers 1–17 ($P > 0.05$).

The measured resistances in conditions A–D can be used to estimate P_K. Fig. 5 shows that P_K in these conditions is closer to 1.5×10^{-6} cm/sec than to $P(\phi_g - \phi_K)$, which according to the discussion above suggests that $\nu_K > 0$. The figure also predicts resistances for conditions H–L, using $P_K = 1.5 \times 10^{-6}$ cm/sec and $P(\phi_g - \phi_K)$. If $\nu_{Na} = \nu_K$, the pump is electroneutral and should not affect membrane resistance according to the model. When $\nu_{Na} = 3$ and $\nu_K = 0$, equation A 10 yields a large effect which would make an accurate choice of P_K difficult, because of the factor $(\nu_{Na} - \nu_K)^2$ in its denominator (cf. Table III).

Relation of Reaction Conductance L_{rr} to Changes in Ionic Concentrations

According to the model and the assumptions of irreversible thermodynamics, L_{rr} may depend on the parameters of state of the system (concentration, temperature,

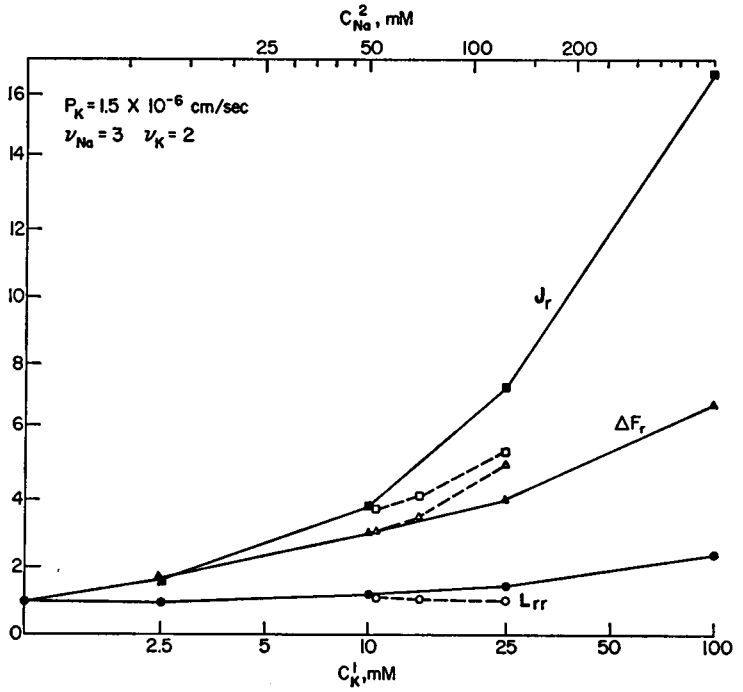

FIGURE 6 Relation of calculated J_r, L_{rr}, and ΔF_r to external K^+ and internal Na^+ for conditions E–L of Table I. $P_K = 1.5 \times 10^{-6}$ cm/sec, $\nu_{Na} = 3$, $\nu_K = 2$. J_r, L_{rr}, and ΔF_r are scaled to their values at $C^1_K = 1$ mM (see text). The filled symbols connected by continuous lines represent H–L (varying C^1_K at $C^2_{Na} = 45$ mM), and the open symbols connected by dashed lines represent E–G (varying C^2_{Na} at $C^1_K = 10$ mM). If $\nu_K = 0$, L_{rr} and J_r are multiplied by about one-third and show the same approximate relation to C^1_K and C^2_{Na}.

pressure) but should be independent of the reaction rate J_r and the driving force $-\Delta F_r$ (Rapoport, 1970; Fitts, 1962). While proof of this independence must await an exact understanding of the mechanism of Na-K transport, the relation of L_{rr} to changes in ionic concentrations can be obtained from the data of this paper. (L_{rr} was assumed constant for small changes when equations A 10 and 3 (below) were derived).

As pointed out, the data in Table I are insufficient to estimate L_{rr} at 1 hr because the electrogenic effect of the pump, H, is insignificant. The change, if any, of L_{rr} with time should be studied at intervals shorter than 1 hr when $\phi_g \ll \phi_K$ and $H \ll 0$, so that the pump's effect on membrane potential remains sizable and errors in estimating ϕ_g and ϕ_K are relatively unimportant in the calculations.

Conditions E–L of Table II have $H \ll 0$ and can be used to estimate L_{rr} for large concentration changes. L_{rr} was calculated to change much less than J_r and ΔF_r for the different assumed P_K's and stoichiometries. Fig. 6 is an example of these calculations when $P_K = 1.5 \times 10^{-6}$ cm/sec, $\nu_{Na} = 3$, $\nu_K = 2$. If $\nu_K = 0$, equation 3 yields values of J_r and L_{rr} which are about three times those in Fig. 6. In the figure, J_r, L_{rr}, and ΔF_r are scaled to their values at $C^i_K = 1$ mM (H in Table II), which are, respectively, 9.4 pmoles/cm² per sec, 12.3×10^{-16} moles/sec per cm² per joule, and -7645 joules. A figure similar to Fig. 6 was obtained for $P_K = P(\phi_g - \phi_K)$, but it gave a value of J_r at $C^i_K = 10$ mM much larger than J_r in Table III or when calculated from Na^+ tracer flux or ATP hydrolysis (see below). These calculations suggest that P_K is closer to 1.5×10^{-6} cm/sec than to $P(\phi_g - \phi_K)$.

L_{rr} as Obtained from Na^+ Tracer Efflux

The relative constancy of L_{rr} in Fig. 6 for C^i_K between 1 and 10 mM suggests that, if such is the case, $J_{active,\,Na}$ (equation 15 *a* of Rapoport, 1970) can be differentiated with respect to ln C^i_K when L_{rr} and ν_i are taken as constant, to give

$$\frac{\partial J_{active,\,Na}}{\partial \ln C^i_K} = (\nu_{Na}\nu_K - \nu^2_{Na})FL_{rr}\frac{\partial \phi_g}{\partial \ln C^i_K} - \nu_{Na}\nu_K L_{rr}RT. \qquad (3)$$

If $\partial\phi_g/\partial\phi_K \simeq 1$, a plot of $J_{Na\,(tracer)}$ efflux (the change in which is taken as $J_{active,\,Na}$ [Keynes, 1954]) against ln C^i_K should be linear with a slope of $-\nu^2_{Na}RT\,L_{rr}$. Data of Sjodin and Beaugé (1968) when plotted in Fig. 7 for C^i_K between 1 and 10 mM, give a straight line with a slope of -9.73 pmoles/cm² per sec. For $2 < \nu_{Na} < 3$, then $4.4 < L_{rr} < 10.0 \times 10^{-16}$ moles/cm² per sec per joule, which agrees with the initial estimates of L_{rr} in Table III and in Fig. 6. For H–L of Table II, when C^i_K is between 2.5 and 100 mM, $\partial\phi_g/\partial\phi_K \simeq 0.85$, a number which does not modify significantly the estimate of L_{rr} from Fig. 7.

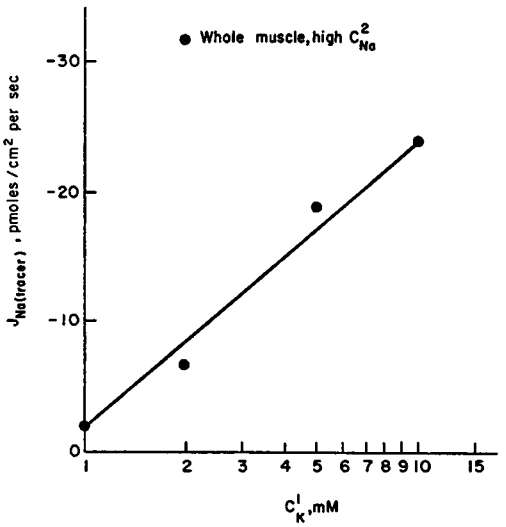

FIGURE 7 Relation of $J_{Na\ (tracer)}$ efflux to ln C^1_K for Na^+-loaded frog sartorius from data of Sjodin and Beaugé (1968). Efflux is defined as a negative flux.

DISCUSSION

The conclusions of this paper are limited because a complete set of relevant observations to test the model was not made in any experiment. It is suggested that the following measurements should be made on individual muscle pairs:

(*a*) At any one time, C^g_K, C^g_{Na}, ϕ_g, and R^o should be obtained. Cross et al. (1965) did not measure R^o which can be used to estimate P_K, nor did they find the individual C^g_{Na}. The use of a mean, $\bar{C}^g_{Na}$, leads to error in calculating $J_{net,\ Na}$ by equation 1 *b*. $J_{net,\ Na}$ calculated by equation 1 *b* can be compared with the value obtained by equation 2 to test the model, if an accurate C^g_{Na} were known.

(*b*) The above measurements should be made at two separate times to obtain dC^g_i/dt and the individual $J_{net,\ Na}$ and $J_{net,\ K}$. Time dependence was not studied by Martirosov and Mykaelian (1970), and Harris and Ochs (1966) did not measure C^g_{Na} with time.

(*c*) Measurements cited above should be made at different initial C^g_{Na} and C^g_K in order to relate L_{rr} and P_K to potential, concentration, and time.

The question of whether K^+ is actively transported ($\nu_K > 0$) could be resolved if an accurate P_K were known in Na^+-loaded muscles. The membrane resistances in conditions A–D, and the L_{rr} and J_r as derived with different P_K's, suggest that P_K is closer to 1.5×10^{-6} cm/sec than to $P(\phi_g - \phi_K)$. This implies, according to Fig. 4, that K^+ is transported into the muscle. Active K^+ transported is also suggested by observations that Rb^+ and Cs^+, like K^+, stimulate active Na^+ efflux, and that both Rb^+ and Cs^+ are actively transported into frog muscle (Adrian and Slayman, 1966;

Beaugé and Sjodin, 1968; but see Harris and Ochs. 1966) as well as in rat muscle (Relman et al., 1957).

Geduldig (1968) showed that ouabain, which inhibits the pump, decreases membrane resistance in Na^+-loaded muscle, which means that $R^o/R > 1$ rather than < 1, as suggested by the model. This discrepancy is important and should be studied further. It could be that ouabain decreases passive resistance, since it increases potassium permeability in rat myometrium (discussed by Taylor et al., 1970), although not in squid axon (Mullins and Brinley, 1969). A way to distinguish R^o from R by means other than metabolic inhibition of the pump would be possible if the time for the pump to respond to a step change in membrane potential were longer than the time required to measure instantaneous resistance R. This would be indicated by a decrease in apparent resistance during the course of application of a current pulse (delayed rectification).

If J_r in Table III decreases exponentially with the time constant $\tau = 30$ min, its average value during the first hour in recovery solution is 57% of its initial value, or between 10 and 16 pmoles/cm² per sec. Under similar conditions, but with dinitrofluorobenzene in the solution (which inhibits ATP synthesis) muscle ATP is hydrolyzed at a rate of 7.7–18.6 mM/kg per hr (Dydynska and Harris, 1966; Harris, 1967). For $r = 40\ \mu$ and for fiber water = 650 cm²/kg wet weight, $J_r = 6$–12 pmoles ATP/cm² per sec over 1 hr and is within the range given in Table III. J_r as calculated from $J_{Na\ (tracer)}$ of Fig. 7, for $\nu_{Na} = 2$ or 3, is between 8 and 12 pmoles/cm² per sec, which also agrees with the above estimates. Thus, the interpretation by the model, that hyperpolarization arises from a reaction-produced current $FJ_r(\nu_K - \nu_{Na})$ which flows across the membrane (equation A 7), leads to an estimate of J_r which agrees with the rate of ATP hydrolysis. It is not necessary to assume that hyperpolarization is caused by K^+ being pumped into the muscle faster than it is replenished in the extracellular space by diffusion from the bathing solution.

In Fig. 6, calculated L_{rr} at $P_K = 1.5 \times 10^{-6}$ cm/sec agrees roughly with the initial L_{rr} of Table III and with the estimate by equation 3 from tracer flux data. Other tracer data in Na^+-loaded muscles cannot be represented by a straight line as in Fig. 7 (Armstrong, 1969), and in unloaded muscles the relation of Na^+ efflux to external K^+ depends on C^I_{Na} (Sjodin, 1970). A nonlinear relation in Fig. 7 would be expected if ν_i or L_{rr} changed with C^I_K, and further experiments in Na^+-loaded muscle are required to test this. Nevertheless, agreement among the L_{rr}'s as obtained from tracer and nontracer observations supports application of the model to Na^+-loaded muscle.

In Table III, for $\nu_{Na} = 3$, $\Delta F_r = -20{,}000$ joules at 1 hr, which is close to the 15,500 joules calculated for the red cell at the stationary state when $\nu_{Na} = 3$, $\nu_K = 2$ (Garrahan and Glynn, 1967). Net Na^+ extrusion is expected to take place for $\Delta F_r < -16{,}000$ to $-25{,}500$ joules as calculated from the "critical energy barrier" of 8370 joules/Na^+ ion, given by Conway (1960). The efficiency of the pump, estimated to

be between 30 and 40% initially (Table III), is comparable to the optimal efficiency of the body as a machine (Brown and Brengelmann, 1965).

Experiments of the type analyzed in this paper give general values for the thermodynamic parameters but say little as regards mechanism. It would be expected that an analysis of deviations about a stationary state would differentiate between linear and nonlinear models. Once this is done, it is expected that the relation between reaction rate and driving force of the reaction, now expressed by equation A 2, can be replaced by a more specific one based upon the mechanism of the active transport pump.

APPENDIX

Summary of Model of Part I (Rapoport, 1970)

The pump is constituted by the net chemical reaction r in the active region of the membrane.

$$\mathrm{ATP} + \nu_{\mathrm{Na}}\mathrm{Na}^2 + \nu_{\mathrm{K}}\mathrm{K}^1 \leftrightarrows \nu_{\mathrm{Na}}\mathrm{Na}^1 + \nu_{\mathrm{K}}\mathrm{K}^2 + \mathrm{ADP} + P_i\,, \qquad (\mathrm{A}\ 1)$$

where the ν_i are net stoichiometric coefficients and the inside of the cell is compartment *2*, the outside compartment, *1* (noted by superscripts). The active Na^+ and K^+ fluxes are, respectively, $-\nu_{\mathrm{Na}}J_r$ and $\nu_{\mathrm{K}}J_r$, where flux is positive from *1* to *2*, and J_r is the rate of the chemical reaction, moles ATP/cm² membrane per sec. We assume that J_r is a linear function of the free energy change of the net reaction, ΔF_r,

$$J_r = L_{rr}(-\Delta F_r), \qquad (\mathrm{A}\ 2)$$

where $L_{rr} > 0$ is a conductance coefficient and

$$\Delta F_r = \Delta\bar{\mu}_p + \nu_{\mathrm{K}}\,\mathrm{RT}\ln C^2{}_{\mathrm{K}}/C^1{}_{\mathrm{K}} - \nu_{\mathrm{Na}}\,\mathrm{RT}\ln C^2{}_{\mathrm{Na}}/C^1{}_{\mathrm{Na}} + (\nu_{\mathrm{K}} - \nu_{\mathrm{Na}})F\phi_2\,. \qquad (\mathrm{A}\ 3)$$

$\Delta\bar{\mu}_p$ is the free energy change in the breakdown of ATP, F is the Faraday, ϕ_2 membrane potential. ΔF_r depends on membrane potential if $\nu_{\mathrm{Na}} \neq \nu_{\mathrm{K}}$ because of the last term in equation A 3. The active membrane current $FJ_r(\nu_{\mathrm{K}} - \nu_{\mathrm{Na}})$ is equal and opposite to the net passive current $\sum_i g_i(\phi_i - \phi_2)$ when net current is zero:

$$I_{\mathrm{net}} = 0 = \sum_i g_i(\phi_i - \phi_2) + FJ_r(\nu_{\mathrm{K}} - \nu_{\mathrm{Na}}), \qquad (\mathrm{A}\ 4)$$

where the g_i are the specific ionic conductances and $\phi_i = z_i\mathrm{RT}/F \ln C^1{}_i/C^2{}_i$ are the ionic equilibrium potentials. The g_i can be calculated from P_i (permeabilities) by use of the constant-field assumption,

$$I_i = g_i(\phi_i - \phi_2) = P_i\frac{F^2\phi_2}{\mathrm{RT}}\frac{C^2{}_i\,\exp\,(z_i\phi_2 F/\mathrm{RT}) - C^1_i}{1 - \exp\,(z_i\phi_2 F/\mathrm{RT})}\,. \qquad (\mathrm{A}\ 5)$$

When $\nu_{\mathrm{K}} \neq \nu_{\mathrm{Na}}$ the pump is electrogenic and the membrane potential differs from the diffusion potential, ϕ_{diff}, by the quantity H, where

$$\phi_{diff} = RT/F \ln \frac{w}{y}, \tag{A 6}$$

and where

$$w = C^1_K + (P_{Na}/P_K)C^1_{Na} + (P_{Cl}/P_K)C^2_{Cl}$$
$$y = C^2_K + (P_{Na}/P_K)C^2_{Na} + (P_{Cl}/P_K)C^1_{Cl},$$

H is given by

$$H = \phi_2 - \phi_{diff}$$
$$= \frac{\sum_i g_i \phi_i}{\sum_i g_i} - \phi_{diff} + \frac{FJ_r(\nu_K - \nu_{Na})}{\sum_i g_i} \tag{A 7}$$

as shown by solving for ϕ_2 in equation A 4 and substituting in the first expression of equation A 7.

For an electrogenic pump, $H \neq 0$ and there is a steady-state passive current $\sum_i g_i(\phi_i - \phi_2)$ given by summing individual ionic currents of equation A 5, where w and y are given by equation A 6,

$$I_{passive} = \frac{F^2\phi_2 P_K}{RT} \frac{w - y \exp(\theta)}{\exp(\theta) - 1}, \tag{A 8}$$

and where we have inserted the contraction $\theta = F\phi_2/RT$. The slope resistance of the passive region of the membrane is $R = -d\phi_2/dI_{passive}$, which is obtained by differentiating equation A 8 with respect to ϕ_2.

$$1/R = \frac{F^3\phi_2 P_K \exp(\theta)}{(RT)^2}\left[\frac{w - y}{(\exp(\theta) - 1)^2}\right] - \frac{F^2 P_K}{RT}\frac{w - y \exp(\theta)}{\exp(\theta) - 1}. \tag{A 9}$$

As pointed out in part I, resistance R^o in the presence of the pump is expected to be less than or equal to resistance R in its absence, if both are measured at the same membrane potential and ionic concentrations. This inequality will obtain if $\partial J_r/\partial(-\Delta F_r) \geq 0$. In order to adjust membrane potential to ϕ_2 when the pump is abolished, for the same ionic concentrations and permeabilities a steady-state current equal to the original pump current must be applied across the membrane by a microelectrode. The distinction between this steady microelectrode current and the original pump current is that the former is independent of ϕ_2, while the latter should not be because of the relation of equation A 3 (J_r depends on ϕ_2). Once a steady microelectrode current is established, an additional applied current $dI_{applied}$ should change membrane potential by $d\phi_2$ so as to give the resistance R of equation A 9, as $dI_{applied} \to 0$. When the pump is not inhibited and produces the steady current which can be changed by membrane potential, the observed resistance is $R^o = -d\phi_2/dI_{applied}$. When L_{rr} and ν_i are constant, it was shown in part I that

$$\frac{R^o}{R} = \frac{1}{1 + RF^2L_{rr}(\nu_{Na} - \nu_K)^2} \leq 1. \tag{A 10}$$

In the absence of a steady pump current (pump absent or electroneutral, where $\nu_K = \nu_{Na}$), $\phi_{diff} = \phi_s$ and equation A 9 takes a simplified form given by equation *6.0* of Hodgkin and Katz (1949). The efficiency of the pump can be described in terms of membrane potential and stoichiometry, as shown in part I. A general expression for it is,

$$\text{Efficiency} = \frac{-J_{\text{active, Na}}(-\Delta\bar{\mu}_{Na}) - J_{\text{active, K}}(-\Delta\bar{\mu}_K)}{J_r(-\Delta\bar{\mu}_p)}, \qquad (A\ 11)$$

where $\Delta\bar{\mu}_i = RT \ln C^1_i/C^2_i + F\phi_s$ for the cations.

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